Potential of biofertilisers to improve performance of local genotype tomatoes

Carmen Puia,[1] Roxana Vidican,[2] Gyöngyi Szabó,[1] Vlad Stoian[2]

[1]Department of Plant Pathology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca; [2]Department of Microbiology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania

Abstract

Complex microbial communities in the plant rhizosphere are responsible for their success in ecosystems. Supplementary inoculation of soil with mycorrhizal fungi and rhizospheric bacteria may act as a plant growth-promoting factor. The present study aims to assess the potential use of biofertilisers on tomato as a way of increasing yield and stability of root exploration area. The experiment was set up in greenhouse, regarding the evaluation of growing dynamics of plants, mycorrhization level and obtained yield. The identification of effective inoculation variants can lead to a standardisation of technologies of growing for local plant genotypes. Data analysis was performed based on the ANOVA test, followed by Tukey HSD, principal component analysis and cluster analysis in order to identify the potential of bioproducts to stimulate the development of tomato plants. Application of bacterial biofertilisers does not stimulate enough the aboveground development of plants. An antagonistic reaction is visible between exogenous mycorrhizas and those specific in soil, acting slightly different for each genotype. Mycorrhizal level in root systems is more dependent on applied biofertilisers than on analyzed genotypes. For the variants without additional fertilisers, a high level of mycorrhization is visible only after 75 days from the transplantation. Based on results we can conclude that microbial active fertilisers may represent viable solutions to increase yield capacity and root exploration area for local tomato genotypes.

Correspondence: Carmen Puia, Department of Plant Pathology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, 400372, Cluj-Napoca, Romania.
E-mail: carmen.puia@usamvcluj.ro

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Introduction

The vesicular-arbuscular mycorrhiza associations (AMF) are the most relevant symbiosis in ecosystems, developed after the synchronised coevolution of plants and fungi in the same environmental conditions (Bonfante and Genre, 2008; Helgason and Fitter, 2009; Montaño *et al*., 2012). The extra radicular hyphae increase the exploitation area of associated plants, which results in an efficient usage of the soil resources, together with the promoted growth and development (Brundrett and Ashwath, 2013; Conversa *et al*., 2012; Garg and Chandel, 2010; Jayne and Quigley, 2014; Koltai and Kapulnik, 2010; Lambers *et al*., 2009). Inoculating the soil had beneficial results in the productive ecosystems, especially when the indigenous mycorrhizal flora is not infectious or efficient enough (Hoeksema *et al*., 2010; Lemanceau *et al*., 2015; Rillig *et al*., 2014). The success of mycorrhizal inoculum depends on the capacity to compete for resources of alien species and the association speed with the rhizospheres plants to which these are introduced to (Garg and Pandey, 2015; Gianinazzi *et al*., 2010; Vicente-Sánchez *et al*., 2014; Watts-Williams and Cavagnaro, 2014; Wezel *et al*., 2014). The AMF symbiosis are reflected over the photosynthetic processes, the nutritive elements absorption and improvement of the soil properties (Fritz *et al*., 2006; Raviv, 2010; Shrivastava *et al*., 2015; Taffouo *et al*., 2014; Treseder, 2013).

The bacterial functional groups are vital components in the soil processes, enhancing the nutrients dynamics (Bashan *et al*., 2014; du Jardin, 2015; Prashar *et al*., 2014). Promoting the plants growth with rhizobacteria is based on the production of growth regulators, protecting the plants over the pathogen attacks and mobilising the nutrients (Ehrmann and Ritz, 2014; Lareen *et al*., 2016; Yang *et al*., 2013). Rhizobacteria sustains the plants in competition for the space inside the ecological niche, nutrients and production of phitohormones with positive impact over the environment (Abbas *et al*., 2014; Ahmed *et al*., 2014; Moënne-Loccoz *et al*., 2015). Currently, a high diversity of symbiotic and non-symbiotic rhizobacteria are included in activities related to crops, with a role in reducing the negative impact of heavy metals, pesticides or soil pH (Dotaniya and Meena, 2015; Matsushita *et al*., 2015; Paul and Lade, 2014; Verma *et al*., 2014). Using the rhizobacteria in promoting the plants growth is restricted by the inoculums complexity, response of genotypes, ecosystem and dimension of pathogens populations (Barrière *et al*., 2014; Fahad *et al*., 2015; Pandya *et al*., 2014; Park *et al*., 2014).

The present study analyzes the potential of biofertilisers with AMF and rhizobacterias as an improvement for above- and below-ground development of local tomatoes genotypes. Measurement of the relationship between the mycorrhizal and rhizobacterial inoculum and plants is performed in conventional and unconventional crop conditions, with the purpose to appreciate the degree of applicability and adaptability to local conditions of bioproducts

with exogenous microflora. Identifying the variants with positive results might lead to a standardisation of local tomato crops with biofertilisers used in the global agriculture.

Materials and methods

Experimental design

The experiment was located in a greenhouse placed in Cluj-Napoca area, Romania (46°46` N / 23°36` E / 363 m altitude), in 3 repetitions, during 2014, with regards to the evaluation of the growing dynamics of plants, the mycorrhization level and the obtained yield. The air temperature was kept between 8°C (night) and 16°C (day) during plant growth in the nursery. After plant transplantation the temperature was maintained at 16°C (night) and 21-22°C (day) from June to August and with a reduction to 16°C during September-October. Relative humidity of air was maintained between 65-75%. In the greenhouse the soil type was a chernozem on which has been applied a basic fertilisation for tomatoes with 20 t/ha manure (Table 1). The experimental design included a combination of 3 X 2 X 3 factors. The trial was organised in a completely randomised design, with four rows/variant, each variant having a length of 2.25 m and a width of 2.64 m, which consist in a 5.94 m2/variant – divided in two equal parts: one for mycorrhizal traits (3 plants for each assay/variant) and one for yield analysis. As plant material were chosen 3 local tomato varieties: *Hostati* (H-Host), *Inimă de bou* (I-Inib) and *Roz* (R-Roz). Plants were sown in nursery on 10 March and prick out at 30 days from this date. The final transplantation in soil was performed when the plants have reached the age of 60 days. The distance between plants on row was 0.33 m and between rows 0.75 m, which correspond to approximately 40000 plants/ha. The weeds were removed by hand. The irrigation of the crop was carried out with a drip hose irrigation system and an average amount of 5-6 l water / plant every 4-5 days throughout the growing season. Treatments done over the vegetation period had 2 crop conditions: conventional (C-Conv - Bravo 500 SC + Ridomil Gold Plus 42.5 Wp) and unconventional (N-Nconv - Bordeaux mixture + Nettle macerate). Each treatment was applied once during the vegetation period. Bravo 500 SC (0.2% concentration) and nettle macerate (9% concentration) were applied at 30 days from the transplantation in the greenhouse. Ridomil Gold Plus 42.5 Wp (0.3% concentration) and Bordeaux mixture (1% concentration) were applied at 60 days from the plant transplantation. There were also 3 fertilisation levels: unfertilised + natural mycorrhization (N-Nf), supplementary mycorrhization (M-Myk) and bacterial inoculum (B-Bact).

Measurements of plants height were done over a period of 50 days, starting with the moment of transplantation. After this period, the differences of heights between variants were no longer significant. Mycorrhizal colonisation and the extent of hyphae in roots were assessed according the formulas proposed by Trouvelot (http://www2.dijon.inra.fr/mychintec/Protocole/Workshop_Procedures.html) and completed with colonisation degree (Cdeg %), described by Stoian *et al*. (2014) as the product between frequency and intensity of colonisation. It have been calculated the arbuscular percentages in mycorrhizal fragments (a %) and in radicular system (A %) and the degree of colonisation (Cdeg %) as factors of mycorrhization. Assessment of the mycorrhizal parameters was performed at 15, 35, 55, 75, 95 and 115 days from transplanting, based on the coloration with blue ink of 60 root segments/ variant (1cm/ segment) and direct microscopy.

Plant growth was analyzed from 10 to 10 days after 20 days from transplantation, and it stopped when most of plants have reached an average size of 1.5 m. Tomatoes were harvested starting at 60 days from transplantation in greenhouse (in July) till the end of vegetation period, at the beginning of October.

Biofertilisers

The biofertilisers were applied at the final transplanting of the plants in greenhouse, when plants had 5-7 leaves.

Mykosoil – a biofertiliser with AMF from GreenBase Company – 200 units of infection/mL of product (www.mykorrhiza.eu). Fungi present in the product were: *Glomus mosseae, G. intraradices, G. clarum, G. monosporus, G. deserticola, G. brasilianum, G. aggregatum, G. etunicatum, G. fasciculatum, Gigaspora margarita*. The applied dose was 10 mL of commercial product/plant.

Bactofil – a biofertiliser produced by Agro.bio Hungary Kft based on *Azospirillium lipoferium, Azotobacter vinelandii, Bacillus megaterium, B. circulans, B. subtilis, Pseudomonas fluorescens, Micrococcus roseus,* contains also macro and micro elements, biosynthetised enzymes by microorganisms, growth stimulators, vegetal hormones and vitamins (www.agrobio.hu). The applied dose was 0.002 mL of commercial product /plant.

Analysis of experimental data

In order to assess the singular and combined impact of experimental factors on above- and below-ground parameters we performed a three-way ANOVA test in StatSoft Statistica. The rest of experimental data was analysed with *vegan* (Oksanen *et al*., 2015), *Hmisc* (Harrell and Dupont, 2015) and *agricolae* (de Mendiburu, 2014) and *cluster* (Maechler *et al*., 2015) packages specific to the RStudio software, version 0.99.879 (RStudio Team, 2015). For identifying the connections between the experimental factors and the development under- and over ground of plants, Pearson type correlations were calculated. The dendrograms were executed separately for the above- and below-ground developments, and the resulted clusters were used as restrictive conditions in Tukey HSD test. For each type of development, above- and below-ground, the principal component analysis (PCA) was performed with the variance calculated for both of the axis.

Table 1. Soil and manure main characteristics.

Soil	pH	Total N (%)	P_{AL} (ppm)	K_{AL} (ppm)	Humus (%)	Texture
	7.03	0.15	58	240	3.87	Clay-loam
Manure	**Dry matter (%)**	**Total N (g kg^{-1})**	**P_2O_5 (g kg^{-1})**	**K_2O (g kg^{-1})**		
	16.7	18.2	17.4	14.8		

Results

Based on the ANOVA results it is visible the strong influence of variety and biofertilisers on above-ground development of tomato plants (Table 2). The variety has a strong impact at the beginning of the growing season (D20 - F = 112.80***) and extremely significant at 50 days after transplanting (F = 4540.4***). Fluctuations in yield are only significantly influenced by variety, instead for the average of fruits this factor is very significant (F = 53.82 ***). Biofertilisers can be found as a very significant influence during the first two growth stages (D20 and D30) and for the fructification potential in the first and third plant floor (Et.1 and Et.3). For all the mycorrhizal parameters biofertilisers plays an important role by significantly influencing their development (F=15.23 – 212.50***). A significant synergic effect of factors is visible only for the combination of variety and treatment (Vt*Tr) and only for the first plant floor and arbuscules in root segments at 15 days from transplantation. These results are consistent with the role of plant growth promotion by active microorganisms contained by biofertilisers, as previous reported and described by Candido *et al.* (2015) and Egamberdieva *et al*. (2015).

The general aboveground and belowground development of plants is strongly correlated with the experimental factors (Table 3). The aboveground development is a character specific to the studied varieties, while the unfertilised variants do not succeed in offering the plants the conditions to reach the potential to grow and produce. Mycorrhizal indicators are correlated negatively with the lack of fertilisation and positively with the supplementary mycorrhization. The vegetative character dendrogram indicates a reduced specificity of plants reaction to the experimental factors (Figure 1). Separating the variants in clusters is due to the interaction between the factors in a higher mean than the one of a single factor, both when dividing in 2 and also in 3 clusters. The steadiest variety is *Hostati*, completely present in a single cluster and maintaining the cluster's integrity even at the dendrogram's split into 3 clusters. The variety *Inimă de bou* has the same reaction to the conventional and unconventional treatments in the lack of fertilisation, and the vari-

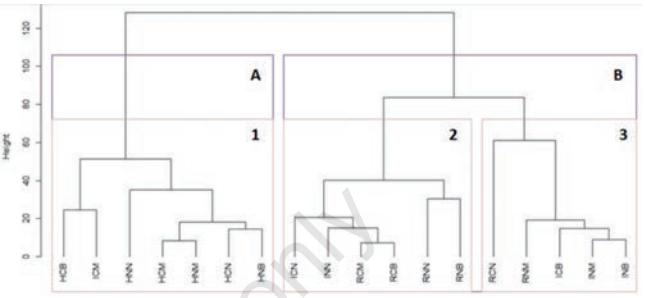

Figure 1. Dendrogram of aboveground development. Legend of code letters is as follows: first letter: H, R, I – plant genotype; second letter: C, N – type of crop system; third letter: N, M, B – type of fertilisation.

Table 2. Results of three-way analysis of variance showing the effect of tomato variety, treatment and fertilisation and their interactions on above- and belowground parameters.

		Average	ANOVA F (P)						
			Vt	Tr	Fe	Vt*Tr	Vt*Fe	Tr*Fe	Vt*Tr*Vt
Aboveground	D20 (cm)	14.34	112.80***	0.05	7.55***	0.78	0.40	3.09	0.75
	D30 (cm)	18.01	61.38***	0.00	7.42***	0.05	0.81	1.30	1.37
	D40 (cm)	18.89	3.57*	0.72	3.52*	1.21	0.56	0.50	1.39
	D50 (cm)	24.41	4540.40***	2.85	0.59	1.73	0.21	0.26	0.52
	Et.1 (%)	66.05	3.92*	1.28	29.15***	4.54*	1.31	0.47	0.37
	Et.2 (%)	68.83	1.90	3.31	9.86	2.98	0.80	0.56	0.76
	Et.3 (%)	59.20	14.11***	0.32	100.49***	1.80	1.00	0.38	0.35
	Prod t ha^{-1}	131.73	5.09*	0.33	4.86*	1.28	0.72	0.20	0.22
	Av.fr (g)	239.29	53.82***	0.02	2.30	1.01	0.90	3.23	0.37
Belowground	Cdeg15 (%)	4.11	0.02	0.58	15.23***	0.27	0.35	0.07	1.21
	a15 (%)	40.50	2.45	3.76	46.14***	3.68*	0.30	0.03	1.27
	A15 (%)	1.95	0.10	0.74	20.88***	1.18	0.04	0.05	1.63
	Cdeg35 (%)	4.21	0.07	0.82	127.74***	1.28	0.22	0.21	0.85
	a35 (%)	46.80	0.83	0.25	212.50***	0.99	1.57	0.02	1.02
	A35 (%)	2.24	0.48	0.23	183.25***	1.74	0.78	0.19	0.48
	Cdeg55 (%)	4.12	1.00	1.34	38.89***	0.22	1.53	1.00	0.23
	a55 (%)	46.45	2.42	0.52	77.50***	0.10	0.97	0.16	0.22
	A55 (%)	2.14	1.80	1.17	51.98***	0.07	1.59	0.79	1.80
	Cdeg75 (%)	5.70	0.43	3.88	89.00***	0.98	1.85	2.01	0.29
	a75 (%)	47.00	0.14	3.05	42.26***	0.09	0.36	0.33	1.12
	A75 (%)	3.02	0.12	4.11	83.02***	0.77	1.41	1.20	0.65
	Cdeg95 (%)	6.42	0.46	0.43	30.89***	0.22	0.17	0.39	1.44
	a95 (%)	48.46	1.75	0.30	19.01***	0.05	0.16	1.17	1.18
	A95 (%)	3.54	1.22	0.00	38.93***	0.47	0.25	0.62	1.08
	Cdeg115 (%)	8.09	2.39	1.25	141.46***	0.06	0.33	0.38	0.78
	a115 (%)	46.52	0.08	1.28	83.61***	1.32	1.48	0.12	0.58
	A115 (%)	4.22	1.94	2.10	156.16***	0.14	0.84	0.48	1.00

ANOVA, analysis of variance; Vt, tomato variety; Tr, treatment; Fe, fertilization; D, increase in height of plants (cm^{-1}) at 20,30,40, 50 days from transplantation; Et 1,2,3, fructification at each plant floor (%); Prod, production (t ha^{-1}); Av.fr, average mass of tomatoes fruits (g); a, A, Cdeg, mycorrhizal parameters (%); 15, 35, 55, 75, 95, 115 – days from plantation. *P<0.05; **P<0.01; ***P<0.001.

ety *Roz* has a similar reaction to bacterial inoculation and mycorrhization overposed to the conventional treatments, respectively bacterial doses and the lack of fertilisation in conditions of unconventional crop. Cluster 1 is characterised by a weak development of plants in the first 30 days after transplantation, with significant differences over the other 2 clusters (Table 4). Starting with 40 days after transplantation, the differences between the variants included in the 3 clusters are reduced and maintained at an insignificant level over the entire vegetation period. At the level of development stages, the differences between the clusters are significant only at stage 3, the variants specific to the cluster 2 having a development plus. The production holds the same constant differences between the clusters at the level of development stage 3, but are not perfectly fit onto the average observed for the fruits weight. The above-ground development of plants is a character imposed especially by the variety and amplified by the applied fertilisations, the total of variance is 81% (PC1 56.21% and PC2 24.83%) in the analysed experimental conditions (Figure 2, Table 5). A specific reaction is noticed for the *Hostati* variety, while the *Roz* and *Inimă de bou* varieties are overlapped as reaction. In the analysed interaction, the variety has the highest impact on the above-ground development.

Variance of mycorrhization parameters highlights the effect of

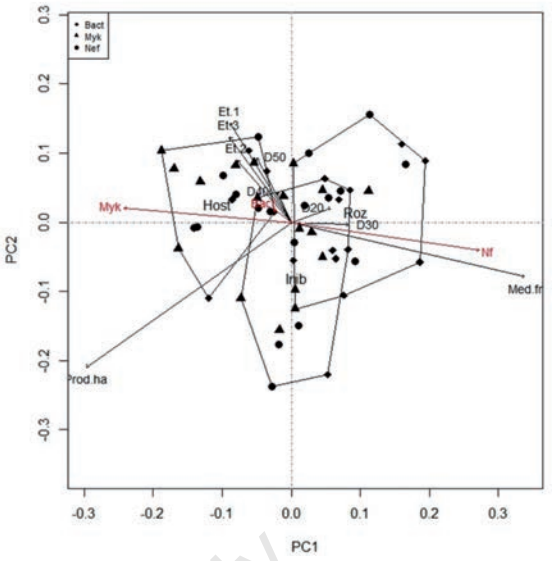

Figure 2. Principal component analysis of interaction between the experimental factors and aboveground development.

Table 3. Correlation of plants development with the experimental factors.

Development		Host	Roz	Inib	Conv	Nconv	Nf	Myk	Bact
Aboveground	D20	-0.80*	0.08	0.71*	-0.02	0.02	-0.19	0.11	0.08
	D30	-0.80*	0.15	0.64*	-0.08	0.08	-0.21	0.16	0.05
	D40	-0.29*	0.11	0.18	-0.17	0.17	-0.29*	0.18	0.10
	D50	0.24	0.09	-0.32*	-0.10	0.10	-0.28*	0.14	0.14
	Et.1	0.26	-0.12	-0.13	0.11	-0.11	-0.71*	0.38*	0.33*
	Et.2	0.02	-0.21	0.18	-0.22	0.22	-0.51*	0.24	0.26
	Et.3	-0.08	-0.23	0.30*	-0.07	0.07	-0.85*	0.40*	0.44*
	Prod	0.00	-0.35*	0.35*	-0.08	0.08	-0.39*	0.24	0.15
	Av.fr	-0.81*	0.44*	0.36*	-0.03	0.03	-0.02	-0.13	0.15
Belowground	Cdeg15	0.03	-0.03	0.00	-0.10	0.10	-0.49*	0.60*	-0.12
	a15	-0.14	0.01	0.13	-0.18	0.18	-0.61*	0.72*	-0.12
	A15	-0.02	-0.02	0.04	-0.11	0.11	-0.48*	0.66*	-0.19
	Cdeg35	0.04	-0.02	-0.01	0.04	-0.04	-0.53*	0.92*	-0.40*
	a35	0.02	-0.06	0.04	-0.04	0.04	-0.65*	0.92*	-0.28*
	A35	0.04	-0.06	0.02	0.01	-0.01	-0.54*	0.93*	-0.40*
	Cdeg55	0.03	-0.12	0.10	0.09	-0.09	-0.46*	0.78*	-0.33*
	a55	0.03	-0.15	0.12	0.03	-0.03	-0.67*	0.82*	-0.16
	A55	0.05	-0.16	0.11	0.07	-0.07	-0.51*	0.81*	-0.31*
	Cdeg75	-0.03	-0.02	0.04	-0.15	0.15	-0.61*	0.84*	-0.25
	a75	0.08	-0.06	-0.02	-0.18	0.18	-0.66*	0.72*	-0.07
	A75	0.00	-0.02	0.02	-0.16	0.16	-0.60*	0.84*	-0.25
	Cdeg95	0.11	-0.09	-0.02	0.05	-0.05	-0.66*	0.65*	0.00
	a95	0.21	-0.19	-0.01	-0.08	0.08	-0.59*	0.55*	0.03
	A95	0.13	-0.15	0.02	-0.01	0.01	-0.64*	0.71*	-0.08
	Cdeg115	0.15	-0.12	-0.02	-0.09	0.09	-0.88*	0.67*	0.21
	a115	0.02	-0.03	0.01	-0.10	0.10	-0.79*	0.72*	0.06
	A115	0.12	-0.12	0.00	-0.10	0.10	-0.83*	0.77*	0.05

D, increase in height of plants (cm^{-1}) at 20,30,40, 50 days from transplantation; Et 1,2,3, fructification at each plant floor (%); Prod, production (t ha^{-1}); Av.fr, average mass of tomatoes fruits (g $fruit^{-1}$); a, A, Cdeg, mycorrhizal parameters (%); 15, 35, 55, 75, 95, 115 – days from plantation. *P<0.05.

Table 4. Stability of clusters for aboveground development.

Cluster	D20	D30	D40	D50	Et1	Et2	Et3	Prod	Av.fr
A1	12.37^{b}	15.16^{b}	18.44^{a}	25.00^{a}	67.51^{a}	69.50^{a}	59.72^{ab}	135.42^{ab}	203.64^{b}
B2	15.29^{a}	19.54^{a}	19.39^{a}	23.87^{a}	64.23^{a}	67.09^{a}	56.12^{b}	118.37^{b}	254.17^{a}
B3	16.11^{a}	21.04^{a}	19.21^{a}	24.18^{a}	66.25^{a}	70.17^{a}	63.12^{a}	143.33^{a}	273.97^{a}

D, height of plants at 20,30,40, 50 days from plantation; Et 1,2,3, specific development at each plant floor; Prod, production (t ha^{-1}); Av.fr, average mass of tomatoes fruits (g $fruit^{-1}$). ^{a,b}Clusters sharing the same letter are not significantly different at P<0.05 in Tukey HSD test.

fertilisers in spatial separation of experimental variants (Figure 3). The supplementary mycorrhization is visible even at the separation in 2 clusters, and at splitting the dendrogram in 3 clusters, it is noticed that each fertilisation represents a homogenous cluster. The similarity between unfertilised control and bacterial inoculation in cluster D is owed to the lack of mycorrhizal inoculum in the fertilisation formula, which sets a reduced development potential of symbiosis compared to the variants in cluster 4. The mycorrhization differences are visible over the monitoring period, but with statistical assurance only concerning the arbuscularity (Table 6). The supplementary mycorrhization acts as a trigger in the first 5 sequences of vegetation and maintains, with slight fluctuations, the percentage of arbuscules in the mycorrhized fragments at approximately 50% over the entire experimental period. Starting with the sequence of 35 days after transplantation, the arbuscularity in fragments increases at 50% in the non-fertilised variants and remain at this level. This phenomenon indicated the mycorrhizal native potential of the soil and the antagonism between the inoculated species and those from the indigenous mycoflora. The exogenous bacteria affect the dynamics of native mycorrhizas and reduces with 10% the arbuscularity in fragments over the entire vegetation period, compared to the unfertilised variants. The arbuscular circuit in the radicular system highlights the strong potential of cellular colonisation of the indigenous mycoflora, the differences being statistically ensured in the middle and the end of vegetation period. The treatments do not manage to increase the arbuscularity levels, bacterial inoculum maintaining this parameter under the 3% level. Although the mycorrhization frequency is of 100%, the colonisation intensity is under 10%, with higher values in the second part of the vegetation period. The differences between the variants are insignificant. Unlike the aboveground development, the mycorrhization of radicular systems is more depended on the fertilisation and less on the variety taken into study (Figure 4, Table 7). The variance explained by the axes sum up 49.32% (PC1 29.59% and

Table 5. Variance explained and factor loadings for aboveground development principal component analysis.

	PC1	PC2	r^2	Pr(>r)	Significance
Host	-0.92	0.40	0.67	0.001	***
Roz	0.99	0.12	0.37	0.001	***
Inib	0.32	-0.95	0.17	0.01	**
Conv	0.57	0.82	0.00	0.933	
Nconv	-0.57	-0.82	0.00	0.933	
Nf	0.99	-0.14	0.12	0.035	*
Myk	-1.00	0.08	0.09	0.078	
Bact	-0.81	0.58	0.00	0.961	

PC, principal component; PC1, 56.21%; PC2, 24.83%. *P<0.05; **P<0.01; ***P<0.001.

Table 6. Stability of clusters for belowground development.

Cluster	a15	a35	a55	a75	a95	a115
C4	47.02^{a}	51.13^{a}	49.65^{a}	49.00^{a}	50.67^{ab}	49.68^{a}
D5	38.97^{a}	50.25^{a}	50.31^{a}	49.81^{a}	53.40^{a}	49.79^{a}
D6	37.83^{a}	40.24^{a}	40.23^{a}	43.15^{a}	42.02^{b}	41.23^{a}
Cluster	A15	A35	A55	A75	A95	A115
C4	1.95^{a}	2.56^{a}	2.25^{ab}	3.65^{a}	4.04^{a}	4.96^{ab}
D5	2.48^{a}	2.64^{a}	2.71^{a}	3.41^{a}	4.36^{a}	5.04^{a}
D6	1.59^{a}	1.60^{a}	1.47^{b}	2.18^{a}	2.37^{a}	2.88^{b}
Cluster	Cdeg15	Cdeg35	Cdeg55	Cdeg75	Cdeg95	Cdeg115
C4	4.55^{a}	4.51^{a}	4.20^{a}	6.74^{a}	6.95^{a}	9.21^{a}
D5	4.22^{a}	4.63^{a}	4.75^{a}	6.21^{a}	7.32^{a}	9.28^{a}
D6	3.68^{a}	3.56^{a}	3.43^{a}	4.44^{a}	5.18^{a}	6.12^{a}

a, A, Cdeg, mycorrhizal parameters; 15, 35, 55, 75, 95, 115 – days from plantation. ^{a,b}Cluster sharing the same letter are not significantly different P<0.05 in Tukey HSD test.

Table 7. Variance explained and factor loadings for belowground development principal component analysis.

	PC1	PC2	r^2	Pr(>r)	Significance
Host	-0.20	0.98	0.10	0.061	
Roz	0.07	-1.00	0.04	0.358	
Inib	0.44	-0.90	0.01	0.72	
Conv	-0.51	0.86	0.07	0.158	
Nconv	0.51	-0.86	0.07	0.158	
Nf	-0.89	-0.46	0.73	0.001	***
Myk	0.94	0.34	0.59	0.001	***
Bact	0.22	0.97	0.02	0.642	

PC, principal component; PC1, 29.59%; PC2, 19.73%. ***P<0.001.

PC2 19.73%). In the absence of fertilisation, strong development of the mycorrhization is spontaneous and it is appearing from the stage of 75 days to the 95 days from the planting. The treatments act as a separation axis between the supplementary and natural mycorrhization, the direction of the two gradients being antagonistic. The correlation between the mycorrhization parameters and the aboveground development is obvious at the analysis of vegetative stages (Table 8). The third stage is significantly correlated with all the mycorrhizal parameters, indifferent of the vegetation phase, which indicates a constant stimulation effect of the symbiotic input. The base stages are strongly influenced by the mycorrhizal value at the end of the vegetation period, and in a reduced level by the values of the intermediary periods (Table 8). The production grows due to arbuscularity in the period 35-55 days from planting and of all mycorrhizal structures at the end of vegetation period.

The average of fruit weights is a variety character and is weakly correlated with the level of mycorrhization, in general the symbiosis slightly decreasing this parameter. The phenomenon is based on the capacity to balance the nutritive elements transfers by the fungus in the plant system and equalising the average of produces fruits.

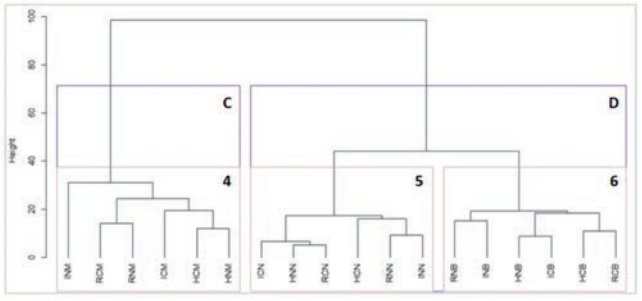

Figure 3. Dendrogram of belowground development. Legend of code letters is as follows: first letter: H, R, I – plant genotype; second letter: C, N – type of crop system; third letter: N, M, B – type of fertilisation.

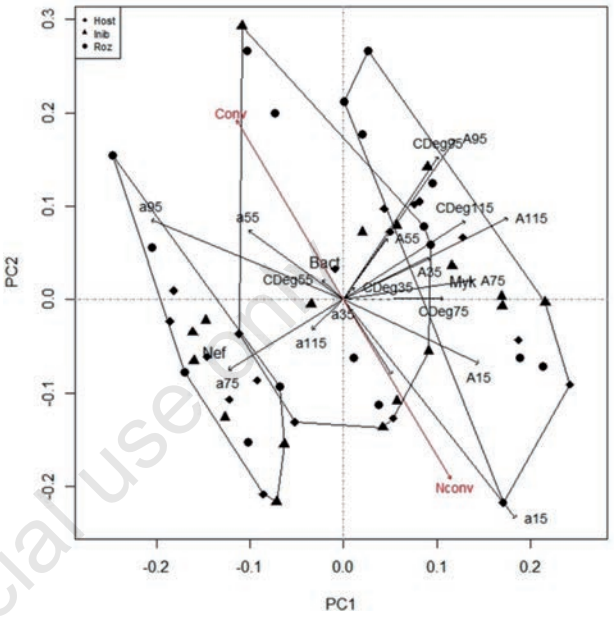

Figure 4. Principal component analysis of interaction between the experimental factors and belowground development.

Table 8. Correlation of above- and belowground development parameters.

	Et. 1	Et. 2	Et. 3	Prod	Av.fr
CDeg15	0.46*	0.23	0.51*	-0.01	-0.08
a15	0.49*	0.39*	0.54*	0.23	0.06
A15	0.42*	0.26	0.48*	0.06	-0.05
CDeg35	0.40*	0.28*	0.46*	0.23	-0.13
a35	0.49*	0.32*	0.55*	0.34*	-0.12
A35	0.42*	0.29*	0.48*	0.27*	-0.15
CDeg55	0.30*	0.33*	0.46*	0.27*	-0.09
a55	0.43*	0.28*	0.65*	0.31*	-0.11
A55	0.34*	0.32*	0.51*	0.29*	-0.12
CDeg75	0.39*	0.41*	0.54*	0.31*	-0.02
a75	0.46*	0.34*	0.59*	0.21	-0.11
A75	0.39*	0.36*	0.53*	0.26	-0.05
CDeg95	0.41*	0.29*	0.51*	0.42*	-0.06
a95	0.50*	0.31*	0.51*	0.31*	-0.18
A95	0.42*	0.33*	0.52*	0.43*	-0.10
CDeg115	0.65*	0.46*	0.77*	0.30*	-0.13
a115	0.60*	0.49*	0.67*	0.38*	0.04
A115	0.63*	0.45*	0.74*	0.31*	-0.10

Et 1,2,3, fructification at each plant floor (%); Prod, production (t ha $^{-1}$); Av.fr, average mass of tomatoes fruits (g fruit^{-1}); a, A, Cdeg, mycorrhizal parameters (%); 15, 35, 55, 75, 95, 115 – days from plantation. *P<0.05.

Discussion

The supplementary mycorrhization and bacterial inoculation act towards stimulating the development of levels 1 and 3 of the plants, as reported before in other studies (Candido *et al*., 2013; Candido *et al*., 2015; Kim *et al*., 1997). Among the tested fertilisers, the bacterial inoculum had the lowest effect over the development with a gradient towards the *Hostati* variety and both of them are integrated in the interaction variety-treatment complex. The mycorrhization and the lack of fertilisation act in different directions, approximately antagonistic.

The use of the mycorrhizal biopreparates stimulates the development of the variety *Hostati* while the *Roz* variety is sensitive in the lack of fertilisation. *Hostati* variety is mostly adapted for productivity, and *Roz* variety for higher weight averages of fruits. For the *Inimă de bou* genotype there are variants which give results in both conditions. This variety, as yield and average weight of fruits, has a moderate reaction towards the experimental factors interactions, located in the center of principal components ordination.

Inoculated bacteria interferes significantly with the symbiosis only in the initial plant development stages, previously reported in several researches (Artursson *et al*., 2006; Hodge and Storer, 2015; Jung *et al*., 2012; Pivato *et al*., 2009). These considerations set the separate analysis of above and under-ground characters, as proposed by Rillig *et al*. (2008), based on reaction of non-AMF genotypes to experimental variables. Separation of the experimental variants in 3 clusters highlights strongly the difference of reactions of the varieties to fertilisation in conventional and unconventional culture system, similar to reported results by Liu *et al*. (2014). Using conventional and unconventional treatments do not set strongly different developments, the 3 varieties having a homogenous response to these actions, as reported by Cavagnaro *et al*. (2006, 2008).

The gradient of supplementary mycorrhization and natural mycorrhization acts in different directions, the level of parameters highlighting the antagonism in the soil. The varieties sensitivity to the experimental factors is higher at *Hostati* and lower for *Roz* and *Inimă de bou*. The inoculated bacteria reduces the level of mycorrhization in the stage of 35-55 days from planting, while the supplementary mycorrhization increases the arbuscularity in the radicular system and the degree of colonisation.

Conclusions

The aboveground development is a character dependent of the variety, while the mycorrhizal development is dependent on the experimental factors. Biofertilisers inoculation is visible in the extent of mycorrhizas in root system, each of them stimulating a specific homogenous development. Exogenous mycorrhizas have a high potential to compete with the indigenous mycorrhizal flora. The exogenous bacteria affects the dynamics of native mycorrhizas and reduces the arbuscularity over the entire vegetation period. Additional mycorrhization leads to reduced values for the degree of colonisation, but produces high values of arbuscules in the fragments colonised, which enhances the transfer of nutrients to plants.

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